nano-scale heterogeneous domains but actual evidence of this link has been vague. Hence where in the phase diagram the live cell membrane is poised would be a very important question in terms of thermodynamics even considering the possible non-equilibrium perturbation to the membrane by actin cytoskeleton or active exchange of components etc. Temperature dependent fluorescence correlation spectroscopy of fluorescent labeled lipids shows a consistently linear trend of mobility as a function of temperature for several different types of lipids. Supported by Monte-Carlo simulation on random walking particles under different states of the system, it shows cell membrane doesn't experience effective miscibility transition well below the physiological temperature. Fluorescence lifetime study with DiI, a known local viscosity reporter, confirms this trend, and shows a clear difference in lifetime between living cell membranes and the blebs derived from them. This also shows even though the membrane blebs are directly induced from the native cell membrane, they may not be under the identical environment which would also suggest two systems being at different positions in the phase diagram.

2161-Plat
Effect of Mechanical Stresses on Lipid Membrane Phase Properties
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We currently investigate how membrane tension modifies the miscibility properties of model lipid bilayers, namely giant unilamellar vesicles. We use fluorescence microscopy to observe coexisting liquid phases within temperature-controlled vesicles under surface tension induced by micropipette aspiration. We measure shifts in liquid/liquid miscibility temperatures as a function of applied tension.
We compare our experimental data to the theoretical prediction of [1], by which a surface tension of ~1mN/m should cause a decrease in miscibility temperature of ~1K. This prediction may explain why Giant Plasma Membrane Vesicles (GPMVs) show large-scale liquid/liquid phase coexistence [2,3] whereas undisturbed cell membranes do not. GPMV's, which are detached from the cytoskeleton, are hypothesized to have lower surface tensions than cell membranes.
[1] Uline, Schick and Szleifer. Changes in phase behavior of lipid bilayers under tension. Submitted.
[2] Baumgart, Hammond, Sengupta, Hess, Holowka, Baird and Webb. Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles. Proc. Natl. Acad. Sci. USA, 104:3165-3170, 2007.
[3] Veatch, Cicuta, Sengupta, Honerkamp-Smith, Holowka and Baird. Critical fluctuations in plasma membrane vesicles. ACS Chem Biol., 3:287-293, 2008.

2162-Plat
Rotational Diffusion of Micrometer-Sized Solid Domains in Lipid Membranes
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The Saffman-Delbrück approximation for translational and rotational diffusion of membrane inclusions [1, 2] is widely used in biophysical studies to relate the inclusion size to the membrane viscosity, but is limited to small inclusion sizes, typically not exceeding 100 nanometers. Although an exact solution of the problem has been derived [3], its computational complexity precludes its practical applications. To overcome this difficulty, we recently developed a simple high-accuracy analytical approximation for the translational diffusion coefficient of a membrane inclusion [4]. Using a similar approach, here we develop a simple and accurate approximation for the rotational diffusion coefficient of a membrane inclusion valid for all combinations of the inclusion size and viscosities of the membrane and surrounding media. We demonstrate the utility of our approximation by using it to analyze our experimental data on rotational diffusion of gel-phase domains on giant unilamellar vesicles showing fluid-gel coexistence.
[1] P. G. Saffman and M. Delbrück, Proc. Natl. Acad. Sci. USA 72 (1975) 3111
[2] P. G. Saffman, J. Fluid Mech. 73 (1976) 593
[3] B. D. Hughes, B. A. Pailthorpe, and L. R. White, J. Fluid Mech. 110 (1981) 349.
[4] E. P. Petrov and P. Schwille, Biophys. J. 94 (2008) L41.

2163-Plat
Membrane Criticality Enriches Components at Sites of Adhesion
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Giant plasma membrane vesicles isolated from living cells undergo large and dynamic critical fluctuations near room temperature. These results have led us to hypothesize that fluctuations persist to growth temperatures of 37°C. In this study, we demonstrate that criticality can greatly impact the organization of components at sites of adhesion between vesicles and a planar supported membrane. Adhesion is accomplished through biotin- streptavidin binding by either directly incorporating trace quantities of biotin-tagged lipids, or indirectly using a biotin conjugated Cholera Toxin B subunit that binds to the ganglioside GM1 incorporated into vesicles. Consistent with previous studies, we observe finite adhesion domains rich in the adhesion molecule (streptavidin) when a PEG lipid is incorporated into vesicles. When vesicle membranes contain coexisting liquid phases, the presence of the adhesion domain affects the localization of phase separated domains based on the partitioning of the adhesion molecules. For example, we observe enrichment of the disordered phase marker DiIC12 at the adhesion domain when the adhesion molecule is a lipid that prefers liquid-disordered phase regions. When vesicles have critical membrane compositions, DiI remains enriched at sites of adhesion at temperatures >15°C above the transition temperature. This occurs even though DiI is not directly involved in adhesion. Instead, DiI partitioning occurs likely because adhesion molecules gather fluctuations that are rich in the lipid probe. At these same temperatures, free-floating vesicles appear uniform on the micron-scale. We also observe DiI enrichment or depletion at the adhesion domain when giant plasma membrane vesicles are adhered to a supported bilayer at 37°C. This occurs even though measured critical temperatures are close to room temperature. Our results demonstrate that membrane criticality can significantly alter the organization of membrane components even when critical temperatures are well below growth temperatures.

2164-Plat
Observation of Inhomogeneity in the Lipid Composition of Individual Nanoscale Liposomes
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Liposomes are one of the most extensively used model systems for studying the physical and chemical properties of membranes and the majority of these studies have implicitly assumed that all liposomes in an ensemble are identical. However recent measurements performed at the level of single liposomes revealed the existence of intrinsic intra-sample heterogeneities that were otherwise averaged out in ensemble experiments.
By employing fluorescently labeled lipids and measuring at the single liposome level we examined the compositional inhomogeneity between individual liposomes within an ensemble. In a recent publication we demonstrated an up to ten-fold variation in the relative lipid composition of individual liposomes with diameters between 50 nm and 15 μm.[1] This observation is made for both a variety of different lipid-labels and compositions, which suggest that compositional inhomogeneity is a general phenomenon present in liposome systems. As a result, bulk measurement of physical and chemical properties that depend on bilayer composition, e.g. phase-transition temperature, will produce results representing the ensemble average, which will be a convolution of the properties arising from many different bilayer compositions of the individual liposomes. Furthermore, we saw that the choice of liposome preparation method greatly influences the degree of compositional inhomogeneity. This could be particularly important when using liposomes as drug delivery carriers where the monodispersity of, and control over, bilayer properties is instrumental.
Since the physicochemical properties of liposomes are directly linked to their composition a direct consequence of compositional inhomogeneities is a polydispersity in the properties of the individual liposomes in an ensemble.
(1) Larsen, J.; Hatzakis, N. S.; Stamou, D. *J. Am. Chem. Soc.* **2011**, *133*, 10685-10687.

2165-Plat
Studying Fusion of Influenza to Supported Lipid Bilayers using Individual Virion Imaging Techniques
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Influenza viruses are membrane-enveloped, negative-strand RNA viruses that employ membrane fusion to release its RNA into host cells and initiate replication. Influenza enters the cell via clathrin-mediated endocytosis. Fusion of the viral and endosomal membrane is facilitated by the conformational change of the viral protein hemagglutinin (HA) at low pH. Traditional bulk fusion assays rely on the fusion fluorescently labeled viruses to synthetic lipid vesicles to obtain kinetic data about the fusion pathway. However, fusion is a stochastic event and only ensemble averages of fusion kinetics are obtained from bulk measurements. To obtain more detail, we use fluorescence dequenching and total internal reflection microscopy (TIRFM) to track and quantify fusion of

individual viruses to supported lipid membranes. Imaging individual virus fusion events enables hemifusion kinetics to be differentiated from pore formation kinetics. Our assays are carried out in high-throughput microfluidic devices where fusion is initiated by reducing the pH in the device. Hemifusion lag times are determined for each individual fusing virus and from this data we can determine hemfusion rate constants (kH) and the number of steps in the hemifusion pathway (N). By using a distinct two-fluorophore labeling approach, we can also measure the time to pore formation of individual viruses following the hemifusion event. In this work, we compare fusion characteristics of different strains of influenza, including seasonal and pandemic strains, and show that there are significant differences in kinetics among them.

2166-Plat
Weak Carbohydrate-Carbohydrate Interactions Measured by Colloidal Probe Microscopy
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The weak calcium mediated interaction between membrane based carbohydrates derived from the marine sponge Microciona prolifera has been investigated by colloidal probe microscopy. An in situ synthesis of glycolipids was employed to measure the dynamic strength of homomeric self-association between sulfated and non-sulfated oligosaccharides. For this purpose an in situ protocol has been developed to entirely rely on a defined synthetic system with adjustable composition and physical properties. Since thermal undulation of membranes renders analysis of binding affinity cumbersome we rely on solid supported membranes with preserved fluidity and minimal non-specific interaction. We investigate the attraction of carbohydrates derived from Microciona prolifera with a focus on the role of calcium ions, the necessity of the sulfate group, membrane fluidity, and loading rate. Membrane fluidity is of particular interest considering that lateral organization of ligand-receptors into small-scale clusters as a result of competition between binding enthalpy and mixing entropy is only possible in laterally mobile matrices.
We found that nanoclusters consisting of 4-8 homomeric bonds were formed in the contact area between colloidal probe and solid supported membrane. Depletion of calcium or removal of the sulfato group resulted both in a disappearance of specific interactions.

Symposium: Soft Lithography for Biology

2167-Symp
Next Generation Proteomics: Towards High-Throughput Protein Analysis via Microfluidic Integration & Soft Materials
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While the genomics revolution has had sweeping impact on our understanding of life processes, the arguably more important ''proteomics revolution'' remains unrealized. Proteins are more directly linked to function than genes, but proteins are also dynamic and more biochemically complex. Consequently, protein analysis often demands multi-stage biochemical assays to measure not one, but multiple physicochemical properties (e.g., Western blot, 2D electrophoresis). Unfortunately, benchtop assays consume significant resources, making the biological sciences protein 'data limited'. To surmount these challenges and realize an era of high throughput proteomics, innovation in instrumentation is needed.
Microfluidic technology has advanced separations science, yet progress in multi-stage separations has lagged. Accepted multi-stage design approaches suffer from inherent information loss owing to strategies that 'discretize' first-stage separations by mapping readouts to discrete compartments in a second-stage. At UC Berkeley, we are introducing novel non-discretizing integration strategies. This talk will highlight multi-stage assays uniquely enabled by our 'μMosaic' fabrication technique: an approach that allows us to regionally photopattern 2D microchambers with heterogeneous, discrete crosslinked polyacrylamide gels. Our design strategy yields low-dispersion, near lossless electrokinetic material transport between disparate assay stages. In one example, I will summarize our recent progress towards fast, hands-free and perhaps even quantitative Western blotting, employed here for analysis of specimens from clinical sample repositories. Our ultimate goal being to advance the understanding of life processes - including development and disease - through quantitative bioinstrumentation.

2168-Symp
Microfluidic Platform for Vascular Biology: Angiogenesis and Anastomosis in Microfluidic Devices
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Angiogenesis occurs within the body in a controlled manner during development, wound healing, and ovulation. New vasculature is also formed in an uncontrolled manner during disease processes such as age-related macular degeneration, atherosclerosis, autoimmune diseases and in response to cancer. High-throughput *in-vitro* techniques that examine the effects of drug candidates on the formation of ''true'' endothelial vessels that are accessible to advanced imaging techniques are needed to increase efficacy and speed development.
This presentation will describe the development of a novel microfluidic device that 1) allow formation of network of perfusable endothelial vessels, 2) observe angiogenesis in response to VEGF stimulation, and 3)observe anastomosis to form a single vessel with a hollow lumen, which can be traversed by beads or red blood cells. Characterization of the vessels through fluorescent immuno-histochemical techniques show that tight-junctions form between the endothelial cells and f-actin is deposited along the length of the vessel. Within each device multiple independent vessels robustly form with the ability to analyze many devices on a single platform and imaged using DIC and confocal microscopy.
Pharmaceuticals can be introduced at different stages of vessel formation and have been shown to inhibit endothelial proliferation, migration and vessel anastomosis in a dose dependent manner. Screening of drug candidates can also be done in our newly developed cancer induced neovasculature indication, where proximally seeded cancer cells induce vessel formation and recruitment.
The development of this device represents a step forward in high-throughput screening for agents that modulate angiogenesis. Accessible by existing imaging and cellular staining techniques, multiple conditions can be tested simultaneously on continuous vessels with hollow lumens that have grown in 3D extracellular matrix.

2169-Symp
Biological Large Scale Integration
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The integrated circuit revolution changed our lives by automating computational tasks on a grand scale. My group has been asking whether a similar revolution could be enabled by automating biological tasks. To that end, we have developed a method of fabricating very small plumbing devices - chips with small channels and valves that manipulate fluids containing biological molecules and cells, instead of the more familiar chips with wires and transistors that manipulate electrons. Using this technology, we have fabricated chips that have thousands of valves in an area of one square inch. We are using these chips in applications ranging from screening to structural genomics to ultrasensitive genetic analysis. However, there is also a substantial amount of basic physics to explore with these systems - the properties of fluids change dramatically as the working volume is scaled from milliliters to nanoliters!

2170-Symp
Engineered Microenvironments: A Materials Approach to the Regulation of Cell Function
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In vivo, local tissue structure defines the cellular environment, constraining how cells interact with surrounding extracellular matrix substrates, neighboring cells, soluble growth factors, and physical forces. These ''microenvironmental'' cues in turn regulate the behavior of individual cells, such as proliferation, differentiation, migration, and suicide. Using engineered microenvironments, we have begun to expose the complex interplay that occurs between adhesion, force, form, and function in cells. For example, cell adhesion to materials (natural or synthetic) is a central regulator of cellular signaling and function, and is characterized by control loops that affect receptor binding to the substrate, cell spreading and flattening against the material, and the active generation of traction forces as cell contract against these adhesions. We show that these control loops are central to cell proliferation, multicellular patterning, and stem cell lineage commitment. I will use our studies to attempt to illustrate 1) the multiple means by which cell-material interactions can control cell signaling and function; 2) the importance of novel engineering and materials approaches to understanding cellular decision making; and 3) opportunities and challenges for how to connect these insights to the ultimate translational objectives set by regenerative medicine.